Biochimica et Biophysica Acta 1778 (2008) 2520–2525

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Molecular organization of 2-(2,4-dihydroxylphenyl)-5,6-dichlor 1,3-benzothiazole in monomolecular layers formed with diphytanoylphosphatidylcholine: A linear dichroism–FTIR study

Mariusz Gagoś *

Department of Physics, University of Life Sciences in Lublin, 20-950 Lublin, Poland

ARTICLE INFO

Article history:
Received 26 February 2008
Received in revised form 15 July 2008
Accepted 30 July 2008
Available online 6 August 2008

Keywords:
2-(2,4-Dihydroxylphenylo)-5,
6-dichlorobenzothiazole
Monomolecular layers
Molecular aggregates
Linear dichroism
Infrared spectroscopy

ABSTRACT

2-(2,4-Dihydroxylphenyl)-5,6-dichlor 1,3-benzothiazole (dHBBT) has very strong antifungal and antitumoral properties in relation to human cancer cells. The aim of this research was to analyze the binding process of dHBBT molecules to the lipid membrane formed from DPhPC at the air–water interface. The effect of dHBBT on the organization of lipid membranes formed with diphytanoylphosphatidylcholine (DPhPC) was studied with the application of monomolecular layer technique, FTIR spectroscopy and linear dichroism–FTIR. On the basis of linear dichroism experiments the mean orientation angle θ between the molecular axis and the normal to monolayer surface was determined. Mean value was calculated at $\theta = 72°$ and indicates a horizontal orientation of dHBBT molecules in the lipid membrane formed from DPhPC. dHBBT molecules have considerable influence on the orientation of DPhPC acyl chains. The mean value of the angle between normal to monolayer surface and the main axis of the acyl chain is approximately 45° for DPhPC, while for the lipid monolayer containing dHBBT it is approximately 18°. Such extreme changes in orientation of acyl chains indicate a clear influence of the relationship on the dynamic and structural properties of the monolayer formed from DPhPC. Biological activity of dHBBT molecules is tightly associated with its molecular organization. The results of the research presented in this work are potentially valuable in respect of the development of pharmacologically active preparations of dHBBT.

1. Introduction

Biologically active compounds such as 2-aryl-1,3-benzothiazoles have anti-proliferative and antifungal properties and research on this subject is being investigated at many research centers around the world [1–4]. This work represents studies on 2-(2,4-dihydroxylphenyl)-5,6-dichlor 1,3-benzothiazole which shows a very strong inhibitive action towards cancer cell lines in humans [5]. The spectroscopic measurements demonstrate that the processes of aggregation of molecules (especially dimerisation) play a significant role in the molecular organization of the compound in the environment of lipid membranes [6].

Electronic absorption and FTIR–linear dichroism spectroscopy were used to study the molecular organization of dHBBT in organic solvents and lipid membranes containing sterols. It was ascertained that dHBBT molecules formed molecular aggregates not only in organic solvents, but also in lipid membranes. Van't Hoff's dependence shows that molecular dimers are most likely. These are also formed in lipid membranes. A clear influence on pretransition ($L_{\beta'} \rightarrow P_{\beta}$, ~35 °C) and the main phase transition ($P_{\beta'} \rightarrow L_{\alpha}$, ~41 °C) in DPPC allows to conclude that dHBBT has a strong influence on the dynamic property of the pure lipid membrane. Molecular dimensions between adjacent chromophores in the dimer formed by dHBBT were calculated using the exciton splitting theory [7,8]. The mean angle between molecule main axis and its dipole transition moment was calculated using linear dichroism studies [6,9]. The results were used to determine the mean value of the organizational angle between the molecular main axis and normal to lipid multilayer surface (61° in DPPC) [6].

Other studies revealed the effect of pH on the aggregation process of dHBBT. The data obtained by FTIR spectroscopy provided information on the influence of particular functional groups on the aggregation process of dHBBT molecules. Molecular area (24 Å^2) occupied by a single dHBBT molecule as well as the aggregation effect were calculated by UV–VIS spectroscopy together with Langmuir–Blodgett technique [10]. Results presented in the paper refer to studies carried out by means of monolayer technique and ATR–FTIR on binding dHBBT to lipid DPhPC membrane.

DPhPC (Fig. 1B) is a lipid mainly used in biochemical studies as well as for the creation of stable lipid bilayers [11]. Studies indicate that a clear effect of the dHBBT molecules influences both the hydrophilic and hydrophobic part of the lipid membrane. A better understanding

* Tel.: +(48 81) 4456899; fax: +(48 81) 4456684.
E-mail address: mariusz.gagos@ar.lublin.pl.

0005-2736/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2008.07.026

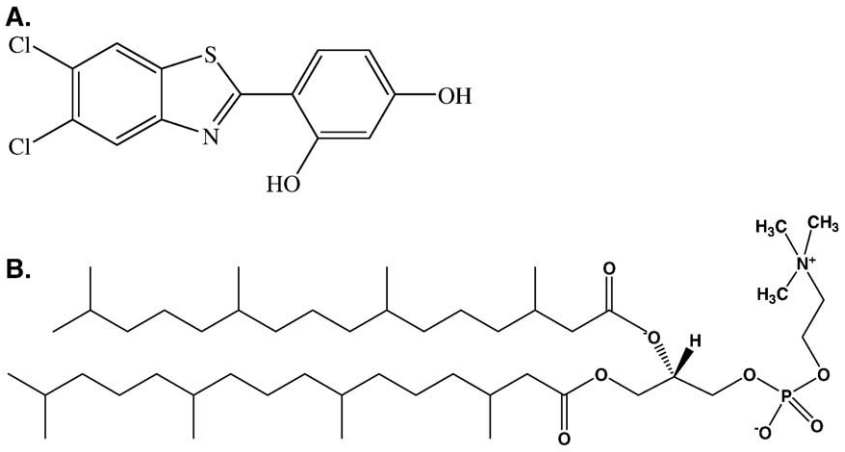

Fig. 1. Chemical structure of dHBBT (A) and diphytanoylphosphatidylcholine (B).

of molecular dHBBT aggregation can have a profound influence on defining the pharmacological application of the drug.

2. Materials and methods

2.1. Chemicals

2-(2,4-Dihydroxylphenyl)-5,6-dichlor 1,3-benzothiazole used in the research (dHBBT, see Fig. 1A) was synthesized at the Department of Chemistry of the University of Life Sciences in Lublin [3,6]. dHBBT (MW=312.17 g/mol) was recrystallized in 96% ethanol and purified by means of HPLC, directly before use. A YMC C-30 column was applied (length=250 mm, internal diameter=4.6 mm) and the solvent mixture CH_3-CN:CH_3OH:H_2O (72:8:3 by volume) was applied as a moving phase. Diphytanoylphosphatidylcholine (DPhPC) was purchased from Avanti Polar Lipids Inc. (USA). Chemicals were stored in argon in a deep-freezer. Ultrapure water was obtained by passing deionized water through Milli-Q equipment (Millipore, Bedford, MA). Solutions of dHBBT were prepared in ultrapure water, and the pH of the solution was adjusted to pH 9 so as to dissolve dHBBT with concentrated KOH solution.

2.2. Monomolecular layers

dHBBT was dissolved in deionized (mQ) water alkalized to pH 9 with KOH and then centrifuged for 15 min at 5000×*g* to remove microcrystals of the drug, still remaining in the sample. A stock solution of dHBBT was adjusted to 1 mg/ml. DPhPC lipids were dissolved in chloroform. Pure lipid monomolecular layer was formed at the air–water interface. Monolayers were formed at the air–water interface and the water subphase was buffered with 10 mM Hepes, pH 7.2. Monomolecular layers were formed in a Teflon trough (282 mm×75 mm) and were compressed along the long side with speed of 15 mm/min. Surface pressure was monitored by a computer-controlled tensiometer, model KSV, Helsinki, Finland. Monomolecular layers were deposited onto a solid support by means of the Langmuir–Blodgett technique (L–B films), with a speed of lift of 5 mm/min at a constant, computer-controlled surface pressure. To remove residuals of water, thin L–B films were placed in a vacuum for 1 h. Before measurements the samples were exposed for 30 min to air conditions (relative humidity 60%) to hydrate components of the monolayers (dHBBT and lipids). Monolayer compression and deposition were carried out at 25±1 °C.

2.3. Infrared absorption measurements (FTIR) and linear dichroism measurements

Infrared absorption spectra were recorded with the Fourier-transform infrared (FTIR) spectrometer, model Bio-Rad FTS 185, equipped with an MCT detector and KBr beam-splitter. Before measurements, the instrument was purged with CO_2-free dry air for 40 min. The attenuated total reflection (ATR) configuration was used with a 10-reflection Ge crystal (45° cut). Typically, 200 interferograms were collected, Fourier transformed and averaged. Absorption spectra in the region between 4000 and 600 cm^{-1}, at a resolution of one data point every 2 cm^{-1}, were obtained using a clean crystal as the background. ATR crystals were cleaned with organic solvents and by "Harrick" Plasma Cleaner for 30 min. Glan-Taylor polarizer was used in the IR polarization experiments. Spectral analysis was performed with Grams 32 software from Galactic Industries.

2.4. Interpretation of FTIR–linear dichroism measurements

Polarized FTIR beam was used to obtain information on molecular order and orientation. The dichroic ratio (*R*) is defined as a ratio of the

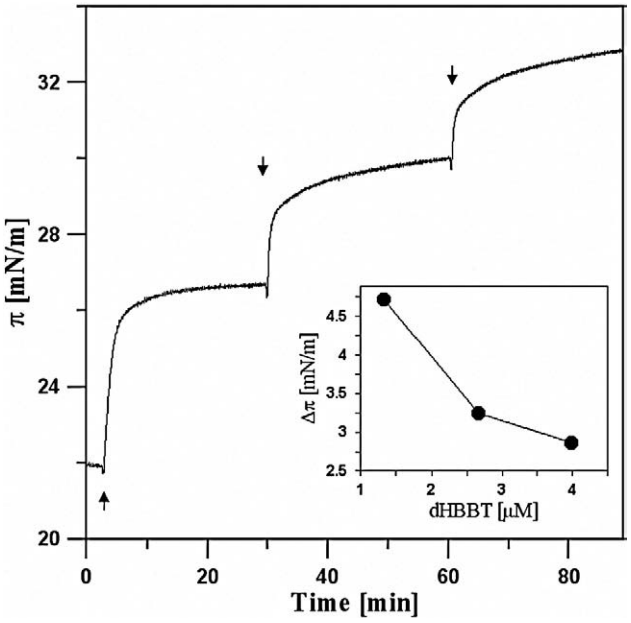

Fig. 2. Surface pressure increase after the injection of dHBBT beneath the monomolecular DPhPC layer at the initial surface pressure of 22 mN/m. Arrows indicate the injection of 10 μl of dHBBT solution into 12 ml of buffer. After each injection the dHBBT concentration in the subphase was increased by 1.33 μM. The solution of dHBBT was prepared in water alkalized with KOH to pH 9. Inset shows the effects of dHBBT injection on the surface pressure changes. Temperature=25 °C.

integrated absorption bands in the parallel, $A_{\parallel}$ and perpendicular $A_{\perp}$ polarization [12]:

$$R = \frac{A_{\parallel}}{A_{\perp}} = \frac{\int A_{\parallel}(v)\mathrm{d}v}{\int A_{\perp}(v)\mathrm{d}v} \quad (1)$$

In practice, because of orientational fluctuations of the molecules in lipid bilayers, an ensemble of molecular orientations contributes to the measured dichroic ratio. Therefore, it is convenient and customary in membrane spectroscopy to analyze polarized IR spectra in terms of the order parameters (S) defined as:

$$S = \frac{(3\langle\cos^2\theta\rangle - 1)}{2} \quad (2)$$

where θ is the average tilt angle of the molecular axis with respect to the axis perpendicular to the plane of the membrane (z axis). On the other hand, S is related to the dichroic ratio by:

$$S = \frac{E^2_x - RE^2_y + E^2_z}{\frac{1}{2}(3\cos^2\alpha - 1)(E^2_x - RE^2_y - 2E^2_z)} \quad (3)$$

where α is the angle between the transition moment of the investigated vibration and the longest axis of the molecule [13–15]. The values of $E^2_x = 1.99$, $E^2_y = 2.13$, $E^2_z = 0.59$ [16,17].

3. Results and discussion

3.1. Monomolecular layers at the air–water interface

Monomolecular layer technique was applied to study the effects of binding dHBBT molecules to the monolayer formed from DPhPC at the air–water interface. Fig. 2 presents the effect of the injection of dHBBT solution into the subphase on the surface pressure increase at the air–water interface. Initial surface pressure of 22 mN/m was selected to be certain that after injection the increase of surface pressure does not extort creation of bilayer or multibilayer (crossing of collapse sp. ~45 mN/m). As expected, only a small fraction of dHBBT molecules from subphase should be able to penetrate the monolayer, taking into account quite high initial surface pressure value (i.e. 22 mN/m). In view of the presented results [6], it is therefore highly probable that after the first injection at those concentrations, the dHBBT molecules remain in the monomeric form in subphase showing surface activity only in relation to lipid membrane. Injection of dHBBT into the subphase in the absence of any lipid film resulted in no surface activity. An increase of surface pressure (approximately 4.7 mN/m) after the first dHBBT injection under the DPhPC monolayer demonstrates the compound binding into the lipid membrane. Subsequent injection of the same dHBBT amounts resulted in decreasing $\Delta\pi$ values. Analysis of the dHBBT compression isotherm shape suggests that, at surface pressures above 20 mN/m, the molecules are mainly able to adopt a vertical orientation in the monolayer [10]. Based on the study of FTIR data presented below, it can be assumed that dHBBT molecules interact both on the surface with heads of polar lipids and with the hydrophobic part of the lipid membrane. Vertical orientation of dHBBT molecules may lead to the aggregation effect in the lipid membrane [10,16].

3.2. FTIR and FTIR–linear dichroism experiments

The monolayers discussed above, were also studied by ATR–FTIR spectroscopy. The monomolecular layers formed from dHBBT containing DPhPC lipid were deposited by Langmuir–Blodgett technique (L–B) on the surface of the Ge crystal (10-reflections). The study was aimed at analyzing the interactions between dHBBT molecules and the lipid membrane. Spectroscopic analysis of the monolayer formed from dHBBT is presented in the study [10]. Fig. 3 presents ATR–FTIR spectra after baseline correction: monolayer formed from pure DPhPC as well as monolayer formed from DPhPC, under whose surface dHBBT

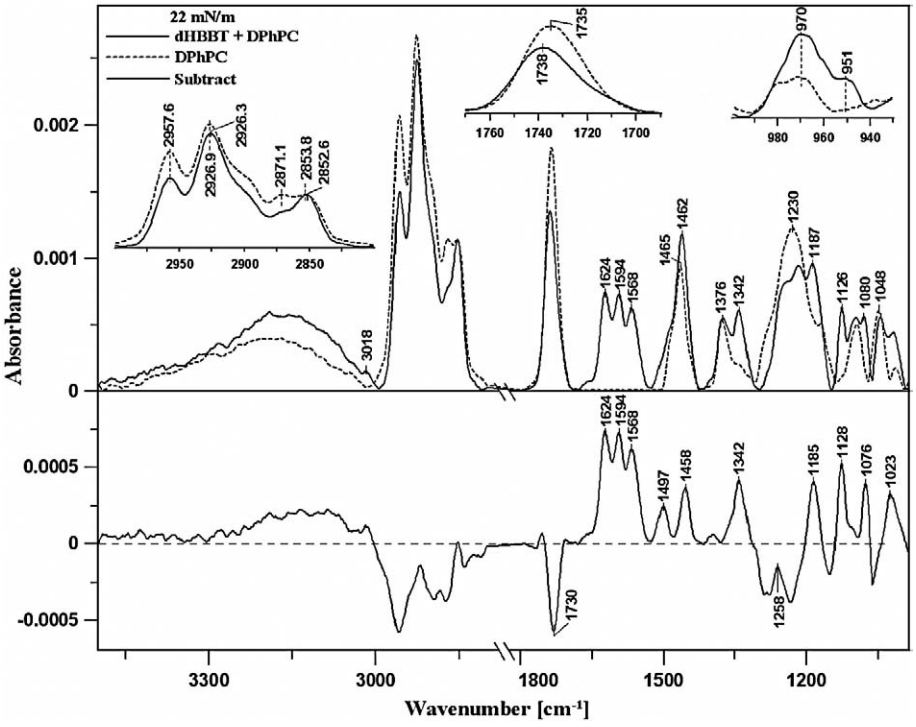

Fig. 3. ATR–FTIR absorption spectra recorded from the Langmuir–Blodgett monomolecular films deposited at two sides of a Ge crystal from the lipid monomolecular layer of DPhPC formed at the air–water interface. Monolayer was compressed and stabilized at the surface pressure of 22 mN/m (dashed line). The DPhPC film was deposited to the crystal after the injection of water solution of dHBBT (pH 9) into the subphase (solid line). Final concentration of dHBBT in the subphase 1.33 μM. Subtracted spectrum: dHBBT in DPhPC minus DPhPC (solid fat line). Temperature = 25 °C

molecules were injected. Within 25 min after dHBBT injection under the surface, the monolayer was deposited onto the surface of the Ge crystal by the L–B technique (surface pressure was stabilized at 22 mN/m). The spectral changes can be observed within the whole range. A wide band within the 1680–1530 cm^{-1} range is associated with the stretching vibrations of the C═N group (1624 cm^{-1}) and the skeletal vibrations of C═C bonds in the plane of ring (1594 and 1568 cm^{-1}) [10]. Wide strong band with maximum at 1230 cm^{-1} corresponds to asymmetric stretching vibrations of the PO_2^- group in the DPhPC. Symmetric vibrations of that group can be found within 1127–1074 cm^{-1}. Binding the dHBBT molecules to lipid monolayer formed from DPhPC caused considerable changes within the discussed ranges, which proves the influence of the compound on the hydrophilic part of the lipid membrane. A spectral shift towards higher frequencies by $\Delta\nu = 3\ cm^{-1}$ of the band of valence vibrations of the C═O bonds (of the monolayer contained dHBBT) is probably connected to the effects of the interaction of the dHBBT molecules with the lipid's ester group [11].

Fig. 4 shows a Gaussian analysis of the parts of the spectrum corresponding to the ν(C═O) vibrations earlier presented in Fig. 3. Panel A presents the spectrum of monolayer formed from pure DPhPC. As can be seen the maximum at 1735 cm^{-1} is a summation of component bands centered at 1740, 1730 and 1713 cm^{-1}. Those maxima are corresponding to *sn*-1, *sn*-2 and hydrogen bonding *sn*-2 carbonyl groups [18–20]. After incorporation of dHBBT molecules into the lipid membrane (panel B) a spectral shift to higher frequencies (1738 cm^{-1}) is observed. This effect could be attributed to the decrease of the absorbance assigned to the *sn*-2 carbonyl group centered at 1730 cm^{-1}. In addition, the increase of absorbance centered at 1713 cm^{-1} probably indicates hydrogen bonding of the *sn*-2 carbonyl group with the dHBBT molecule (C═O···HO–). A more precise explanation may be attributed to the polarity effect, assuming that the *sn*-2 carbonyl group is closer to the PO_2^- group. Interaction of dHBBT molecules with PO_2^- group was observed as changes in band with a maximum at 1230 cm^{-1}. It is probable that the localization of dHBBT molecules in the polar zone of the membrane also has influence on the *sn*-2 carbonyl group. Simultaneously we observed two absorption bands in the region characteristic for asymmetric stretching vibrations of N-CH_3 in the choline group at 951 and 970 cm^{-1} (inset in Fig. 3). In all

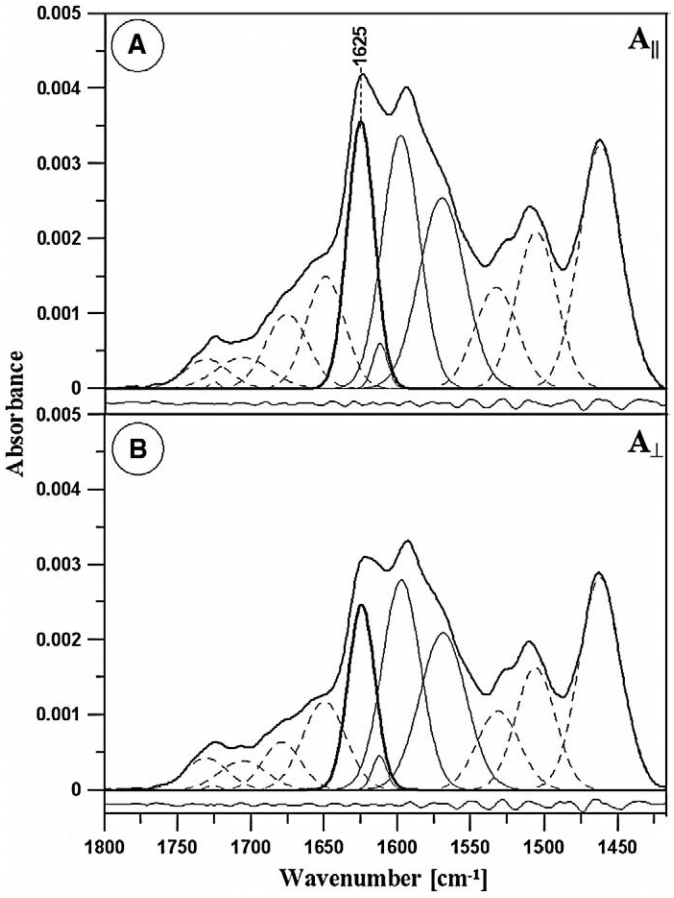

Fig. 5. Polarized ATR–FTIR absorption spectra recorded from the Langmuir–Blodgett monomolecular films of dHBBT deposited on two sides of a Ge crystal. The films were deposited from the monomolecular layer of dHBBT at the air–water interface at the surface pressure of 25 mN/m. The spectrum recorded with parallel polarization of the electric vector of radiation with respect to the plane of incidence is plotted in panel A and the perpendicular polarization in panel B. To perform an accurate curve fitting of the spectra a Gaussian function had been applied. Integrated spectral band (solid fat line) corresponding to the ν(C═N) vibration. The residuals are reported at the bottom of each panel. Temperature = 25 °C.

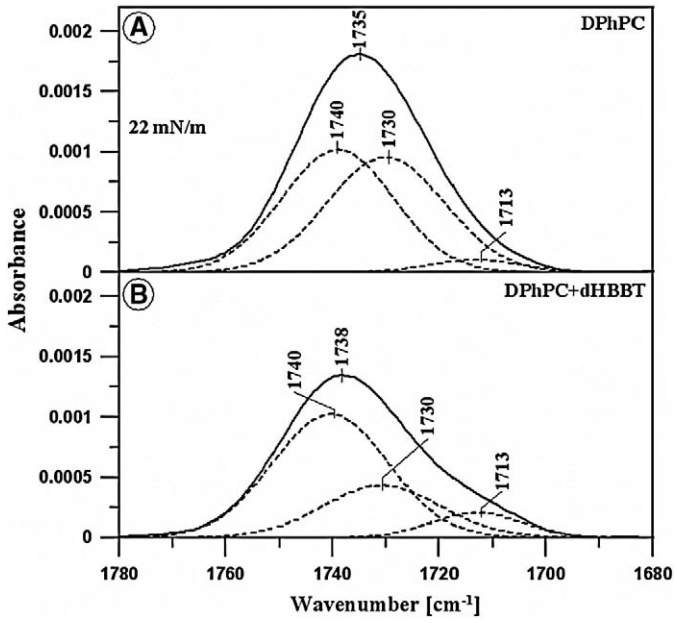

Fig. 4. ATR–FTIR absorption spectra of the ν(C═O) region recorded from the Langmuir–Blodgett monomolecular films of DPhPC (A) and DPhPC containing dHBBT (B; see Fig. 3).

likelihood dHBBT molecules interact with both the phosphate and choline group. The *sn*-1 group is more deeply buried in the hydrocarbon chain matrix.

Symmetric and asymmetric stretching vibrations of CH_2 and CH_3 in the lipid's acyl chains can be observed as intensive bands with maxima within the 2800–3000 cm^{-1} range. Peaks at 2957.6 and 2871.1 cm^{-1} have lower intensities, which can be associated with stretching asymmetric and symmetric vibrations of CH_3 in spectrum of dHBBT incorporated into the lipid monolayer. Spectral shifts of the bands of symmetric and asymmetric stretching vibrations of CH_2 are observed for DPhPC monolayer containing dHBBT [11]. For monolayer formed from pure DPhPC, $\nu_s = 2853.8\ cm^{-1}$ and $\nu_{as} = 2926.9\ cm^{-1}$. After incorporation of dHBBT to the DPhPC monolayer $\nu_s = 2852.6\ cm^{-1}$ and $\nu_{as} = 2926.3\ cm^{-1}$ (measure wavenumber accuracy ~0.9 cm^{-1}).

The shift to lower frequency is also representative for more tightly packed tails. The above-discussed results may lead to the conclusions on the apparent influence of dHBBT on the hydrophobic core of the lipid membrane as well. These results confirm studies carried out earlier, indicating the effect of the compound on dynamic properties of the lipid membrane [6]. The increase in absorbance of the broad band in the region 3000–3300 cm^{-1} in the spectrum of the monolayer formed from DPhPC containing dHBBT (Fig. 3) suggests the possibility of the formation of hydrogen bonds of the compound in the lipid membrane. Most probably the association process between dHBBT

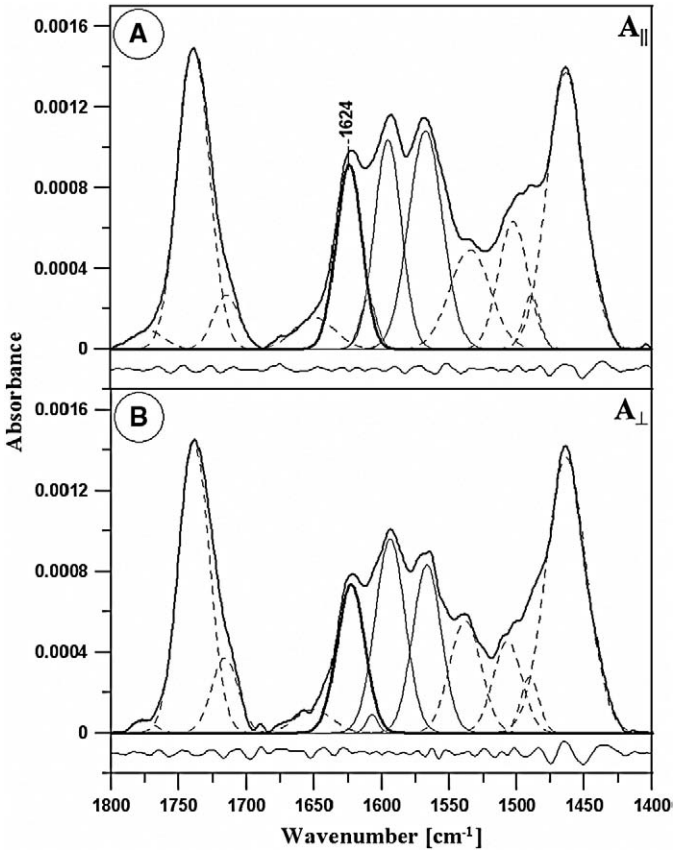

Fig. 6. ATR–FTIR absorption spectra recorded from dHBBT containing the Langmuir–Blodgett monomolecular films deposited from the lipid monomolecular layers at the air–water interface. Monolayer was compressed to the surface pressure of 22 mN/m composed in the same condition as in Fig. 3. The electric vector of the IR radiation was polarized parallel (A) and perpendicular (B) with respect to the plain of incidence. The Langmuir–Blodgett monomolecular films were deposited from the lipid monolayers 25 min after the injection of dHBBT into the subphase. Integrated spectral band (solid fat line) corresponding to the ν(C═N) vibration. The residuals are reported at the bottom of each panel. Temperature = 25 °C.

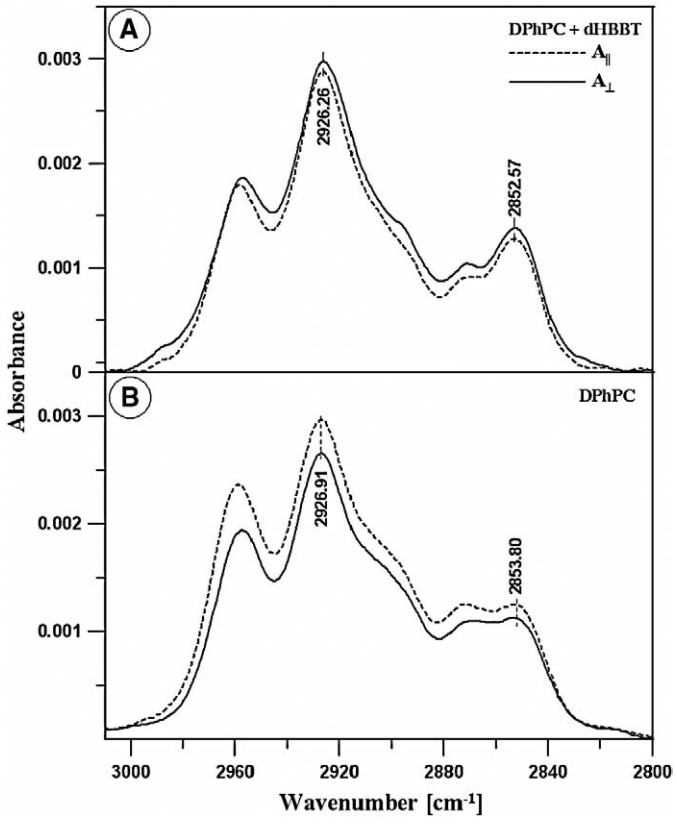

Fig. 7. Polarized ATR–FTIR absorption spectra recorded from the Langmuir–Blodgett monomolecular films deposited at two sides of a Ge crystal from the lipid monomolecular layers (the same condition as in Fig. 3): DPhPC formed at the air–water interface, compressed to the surface pressure of 22 mN/m (B) and the DPhPC film deposited to the crystal after the injection of dHBBT into the subphase (A). Spectra were recorded with polarization: perpendicular (solid line) and parallel (dashed line). The spectral region presented corresponds to the stretching vibrations of CH_3 groups (ν_s 2872 cm^{-1}, ν_{as} 2958 cm^{-1}) and CH_2 groups (ν_s 2853 cm^{-1}, ν_{as} 2927 cm^{-1}).

molecules and also dHBBT–lipid interaction (e.g., lipid ester C═O···HO– from the *para* position in resorcinol ring) may be important in the molecular organization of dHBBT molecules in lipid membranes. This is also confirmed by the band at 1342 cm^{-1} responsible for bending vibrations of the –OH group (absent in the spectrum of the pure lipid membrane).

ATR–FTIR linear dichroism measurements provided information on the orientation of dHBBT molecules in DPhPC membranes. Fig. 5 presents the polarized IR absorption spectra of monomolecular layers formed from pure dHBBT, deposited by the L–B technique on the ATR support. The spectra underwent curve fitting with the same initial set of Gaussian components. As can be seen this component centers at 1625 cm^{-1} and is responsible for the band of valence vibrations of the C═N in the dHBBT ring. The monolayer had been deposited at surface pressure close to 25 mN/m to assure vertical orientation of the dHBBT molecules at the support. This assumption is supported by the isotherm of compression of the monomolecular layer formed with pure dHBBT. At surface pressure of more than 20 mN/m, dHBBT molecules are oriented perpendicular to the surface of the water and create a solid state film [10]. Assuming the same arrangement on crystal surfaces also, it can be accepted that the mean value of an angle between the molecule's main axis and normal to crystal's surface is $\theta = 0°$. The dichroic ratio (Eq. 1) resulting from the integration of the Gaussian component for the band with maximum at 1625 cm^{-1} and associated with stretching vibrations of C═N bond in dHBBT molecule (Fig. 5) is $R = 1.232$. Applying the theory expressed by Eqs. (2) and (3), a mean value of the angle $\alpha = 58.1° (\pm 4°)$ may be calculated, between the transition moment of the investigated C═N vibration and the longest

Table 1
Orientation angle (θ) of the long molecular axis of dHBBT bound to monomolecular layers formed with DPhPC, with respect to the axis normal to the plane of the film, determined on the basis of linear dichroism measurements (see Fig. 5) and Eq. (2)

dHBBT in	Vibration	Position (cm^{-1})	Dichroic ratio R	Order parameter S	α (degrees)	θ (degrees)
DPhPC	ν(C═N)	1624	1.15	−0.35	46	72

±SD: S (±0.05), α, θ (±4°).

Table 2
Mean orientation angle (θ) of the molecular axis along the CH_2 acyl chains of DPhPC in monomolecular layers formed with DPhPC, with respect to the axis normal to the plane of the film, determined on the basis of linear dichroism measurements (Eq. 3)

Membrane composition	Vibration $\nu(CH_2)$	Band position (cm^{-1})	Dichroic ratio R	S	α (degrees)	θ (degrees)
DPhPC	s	2853.80	1.116	0.259	90	44.6
	as	2926.91	1.121	0.244		45.2
DPhPC + dHBBT	s	2852.57	0.959	0.872		17.0
	as	2926.26	0.966	0.837		19.2

The table also presents the orientation angle in the case of the lipid films deposited from monolayers after injection of 1.3 μM dHBBT into the subphase (deposited after time required for equilibration of the system). Angle α has been arbitrary taken as 90° according to the literature data [21].
±SD: S (±0.03), θ (±2°).

axis of the molecule. By the same equations (Eqs. 2 and 3) it is also possible to calculate the average tilt angle θ of the molecular axis with respect to the axis perpendicular to the plane of the membrane [16].

Fig. 6 presents polarized IR absorption spectra of the monolayer formed from DPhPC containing dHBBT (the same conditions as in Fig. 3). Monolayers were deposited by the L–B technique at surface pressure stabilized by 22 mN/m. Analysis of spectrum in the 1800 to 1400 cm^{-1} range for mixed monolayers of lipid with dHBBT revealed changes in the intensity and shape of bands within the range. Calculation of the dichroic ratio for the C═N vibration band of Gaussian component placed at 1624 cm^{-1}, permits the calculation of the mean value of θ angle between dHBBT main molecular axis and normal to surface of the lipid membrane. Results from these calculations are presented in Table 1. Average angle θ=72° (±4°) for dHBBT molecules oriented in lipid monolayer [6].

Based on the presented results, it can be assumed that dHBBT molecules are organized in lipid membranes both on its surface as well as within the hydrophobic core. An effect of dHBBT on alkyl chains of DPhPC can also be observed in the spectral region between 2800 and 3000 cm^{-1}, corresponding to the C–H stretching vibrations of the methyl and methylene groups. Polarized IR absorption spectra of monolayers formed from pure DPhPC and DPhPC containing dHBBT (total concentration 1.33 μM) can be seen in Fig. 7. For the purpose of the investigation only the most intensive band corresponding to the symmetric and asymmetric stretching vibrations of CH_2 groups (band positions presented in the Table 2) was taken. The average orientation angle θ, corresponds to the angle of the cone in which the segmental motions of the lipid acyl chains take place (in principal the *trans-gauche* isomerization). For the symmetric and asymmetric CH_2 stretching vibrations α=90° (in this case α is the average angle between the transition moment of the investigated CH_2 vibration and the longest axis of the acyl chain) [12,21].

Table 2 presents the results of the linear dichroism determinations of molecular order in the lipid monolayers. As can be seen, mean θ value is approximately 45° (±2°) for pure monolayer, and approximately 18° (±2°) for lipid monolayer containing dHBBT [16,17]. Such changes in lipid's acyl chains orientation indicate that dHBBT significantly affects the dynamic and structural proprieties of lipid membranes. It is concluded that dHBBT molecules organized in the lipid phase exert a pronounced ordering effect with respect to the acyl lipid chains. Interaction of dHBBT with a lipid matrix is based upon van der Waals interactions between the acyl chains of the lipid and the drug molecules.

Acknowledgements

The author is indebted to Prof. Andrzej Niewiadomy, head of the group from the Department of Chemistry of the University of Life Sciences in Lublin for dHBBT samples and Dr. Mauro Dalla Serra, head of the group at the FBK-CNR Institute of Biophysics, Unit at Trento (Italy) for making scientific equipment and chemicals in his laboratory available.

References

[1] J. Matysiak, A. Niewiadomy, G. Macik-Niewiadomy, T. Kornillowicz, Dependence of fungistatic activity of 2,4-dihydroxythiobenzanilides on the structure and lipophilic nature of the compounds, Eur. J. Med. Chem. 35 (2000) 393–404.
[2] P. Magdolen, P. Zahradnik, P. Foltinova, Synthesis and antimicrobial activity of new 2-phenylethynylbenzothiazoles and related salts, Arzneimittelforschung 50 (2000) 1023–1027.
[3] S.D. Paranjape, A.S. Bobade, B.G. Khadse, Synthesis and evaluation of new triazolobenzothiazole derivatives as potential anti-tubercular agents, Arzneimittelforschung 51 (2001) 916–919.
[4] H.M. el-Shaaer, S.A. Abdel-Aziz, H.A. Allimony, U.F. Ali, R.M. Abdel-Rahman, Synthesis and antimicrobial activities of some new 2-substituted benzoxazole/benzothiazole derivatives, Pharmazie 52 (1997) 585–589.
[5] A. Niewiadomy, G. Grynkiewicz, W. Szelejewski, A. Kutner, J. Ramza, Derivatives of 2-(b-rezolcylo)-1,3-benzothiazol, the methods of synthesis and containing them pharmacological preparations (in Polish), Patent Pending P-3555900s: Poland (2002).
[6] M. Gagoś, A. Niewiadomy, W.I. Gruszecki, Molecular organization of the antifungal and anticancer drug 2-(2,4-dihydroxyphenylo)-5,6-dichlorobenzothiazole (dHBBT) in solution and in lipid membranes studied by means of electronic absorption spectroscopy, J. Photochem. Photobiol. B. 76 (2004) 33–40.
[7] J. Parkash, J.H. Robblee, J. Agnew, E. Gibbs, P. Collings, R.F. Pasternack, J.C. de Paula, Depolarized resonance light scattering by porphyrin and chlorophyll a aggregates, Biophys. J. 74 (1998) 2089–2099.
[8] W.I. Gruszecki, M. Gagoś, P. Kernen, Polyene antibiotic amphotericin B in monomolecular layers: spectrophotometric and scanning force microscopic analysis, FEBS Lett. 524 (2002) 92–96.
[9] W.I. Gruszecki, M. Gagoś, M. Herec, Dimers of polyene antibiotic amphotericin B detected by means of fluorescence spectroscopy: molecular organization in solution and in lipid membranes, J. Photochem. Photobiol. B. 69 (2003) 49–57.
[10] M. Gagoś, G. Menestrina, A. Niewiadomy, W.I. Gruszecki, Molecular organization of the antifungal and anticancer drug 2-(2,4-dihydroxyphenylo)-5,6-dichlorobenzothiazole in solution and in monolayers: an effect of pH, J. Photochem. Photobiol. B. 80 (2005) 101–106.
[11] D.R. Gauger, H. Binder, A. Vogel, C. Selle, W. Pohle, Comparative FTIR-spectroscopic studies of the hydration of diphytanoylphosphatidylcholine and-ethanolamine, J. Molec. Struct. 614 (2002) 211–220.
[12] L.K. Tamm, S.A. Tatulian, Infrared spectroscopy of proteins and peptides in lipid bilayers, Q. Rev. Biophys. 30 (1997) 365–429.
[13] S.A. Tatulian, L.R. Jones, L.G. Reddy, D.L. Stokes, L.K. Tamm, Secondary structure and orientation of phospholamban reconstituted in supported bilayers from polarized attenuated total reflection FTIR spectroscopy, Biochemistry 34 (1995) 4448–4456.
[14] N.J. Harrick, Internal Reflection Spectroscopy, Harrick Scientific Corporation, Ossining, NY, 1979.
[15] G. Menestrina, Use of Fourier-transformed infrared spectroscopy (FTIR) for secondary structure determination of staphylococcal pore-forming toxins, in: O. Holst (Ed.), Bacterial Toxins, Methods and Protocols, Humana Press, Totowa, New Jersey, 2000, pp. 115–132.
[16] M. Gagoś, J. Gabrielska, M. Dalla Serra, W.I. Gruszecki, Binding of antibiotic amphotericin B to lipid membranes: monomolecular layer technique and linear dichroism–FTIR studies, Mol. Membr. Biol. 22 (2005) 433–442.
[17] A. Sujak, M. Gagos, M. Dalla Serra, W.I. Gruszecki, Organization of two-component monomolecular layers formed with dipalmitoylphosphatidylcholine and carotenoid pigment canthaxanthin, Mol. Membr. Biol. 24 (2007) 1–11.
[18] R.N. Lewis, R.N. McElhaney, Studies of mixed-chain diacyl phosphatidylcholines with highly asymmetric acyl chains: a Fourier transform infrared spectroscopic study of interfacial hydration and hydrocarbon chain packing in the mixed interdigitated gel phase, Biophys. J. 65 (1993) 1866–1877.
[19] R.N. Lewis, R.N. McElhaney, W. Pohle, H.H. Mantsch, Components of the carbonyl stretching band in the infrared spectra of hydrated 1,2-diacylglycerolipid bilayers: a reevaluation, Biophys. J. 67 (1994) 2367–2375.
[20] W. Hubner, A. Blume, Interactions at the lipid–water interface, Chem. Phys. Lipids 96 (1998) 99–123.
[21] H. Binder, T. Gutberlet, A. Anikin, Biaxial ordering of terminal diene groups in lipid membranes: an infrared linear dichroism, J. Molec. Struct. 510 (1999) 113–129.